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OXFORD MEETING OF THE SOCIETY
FOR GENERAL MICROBIOLOGY
24-25 September 1970

By GEORGE A. HOTTLE

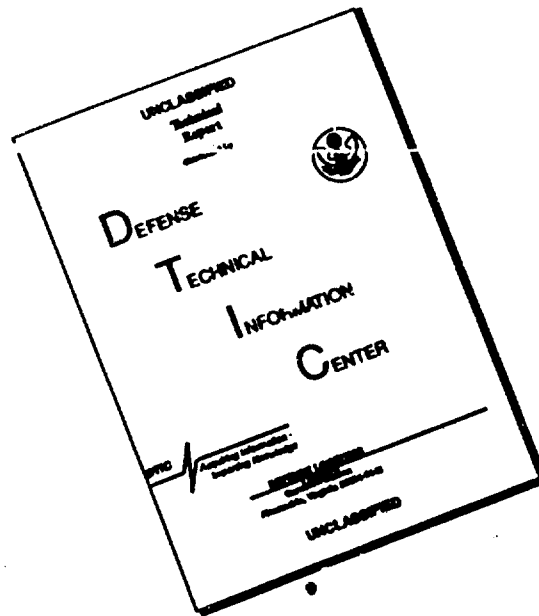
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OXFORD MEETING OF THE SOCIETY FOR GENERAL MICROBIOLOGY
24 - 25 September 1970

INTRODUCTION

Each year the Society holds general meetings in January, April and September to bring together scientists, teachers and students interested in various aspects of the science of Microbiology. The September meeting featuring a Symposium on the Function and Metabolism of Nucleic Acids was held in the Lecture Hall of the University Museum on Parks Road, Oxford. This time was chosen for the meeting at the University because the fall term had not yet opened and full use of student facilities for meals and lodging was permitted at St. Catherine's College. Accommodations for 180 people had been arranged for this meeting. Nevertheless, it was evident that more than that number were present during the first day's (Symposium) sessions. The program for the second day comprised original papers which were presented in two concurrent series of sessions.

SYMPOSIUM

The Symposium provided the most interest because it consisted of accomplished speakers, and enough time was permitted (45 minutes for each) so that the subjects could be discussed in a balanced way. The sessions, on the second day, were arranged so that ten 15-minute presentations were made in each three-hour sitting with a 30-minute break after the first five speakers. This arrangement provided a convenient break for private contact with some of the speakers.

Prof. K. Burton (Department of Biochemistry, University of Newcastle upon Tyne) opened the Symposium by discussing the "Effects of Purine Nucleotide Concentrations on RNA Synthesis in *Escherichia coli*." He carried out experiments with mutant strains of *E. coli* to show the effect of various purines such as hypoxanthine, xanthine, adenine and guanine on the synthesis of nucleic acids. He did this by using radioactive nucleotides in purine-free media and measuring the amount of radioactive nucleic acid which formed when the various strains of *E. coli* grew. He showed that RNA synthesis appears to be more dependent upon changes in the amount of GTP* than upon the level of ATP* in the cells. Some of these strains converted hypoxanthine, xanthine or guanosine to GTP but not to ATP. When adenine nucleotides were in excess, they were converted to guanine nucleotide also.

Within on minutes after the antibiotic rifampicin was added, synthesis of RNA, as measured by incorporation of C¹⁴-labeled uracil, was stopped.

* GTP - guanosine triphosphate
* ATP - adenosine triphosphate

Thus, the RNA chain growth was stopped even though chain initiation by ATP (as shown by other workers) was possible. On adding either adenine or hypoxanthine to a purine-starved strain of *E. coli* the synthesis of stable RNA is restored to normal within four minutes at 25° C. Using other mutants, Burton showed that amino acid starvation of the bacteria has an effect similar to purine starvation, in selectively inhibiting the synthesis of stable RNA. This indicates that different mechanisms are operating for control of RNA synthesis. Amino acid starvation acts primarily upon initiation of stable RNA, and when the amino acids are restored, RNA synthesis is resumed more slowly than when purines are restored after purine depletion.

The second presentation was by Dr. J.D. Smith (Medical Research Council, Laboratory of Molecular Biology, Cambridge) on the "Suppressor Transfer RNAs and their Use in the Study of tRNA Structure and Function" (in strains of *Escherichia coli*). By sequence analysis of the bases in RNA, Smith was able to study the effect of mutation on the change in coding properties of specific RNAs. For instance, he had one mutant identified as SU⁻ -- which carried the codon UAG for translation into the synthesis of tyrosine. By selecting mutants of this strain he was able to get an SU⁺ mutant which now contained the codon CUA which transcribed for the same amino acid.

In another series of experiments, mutants of the SU₁₁₁ tRNA gene were used to study the relationship between nucleotide tRNA structure and functions. With one mutant the base pair GC was changed to AU without changing the functions of the gene. The original mutant grew at 32° C but not at 37° C or 42° C. After three further mutations, by first selecting one which grew at all three temperatures, then another which grew only at 32° C and 37° C, finally, a third which again grew only at 32° C, the base pair had now been changed to AU. Yet, it directed metabolism in the same way that the base pair GC did. Fingerprints of various mutants were obtained on polyacrylamide gel in order to identify the individual nucleotide constituents of nucleic acid.

One of Smith's co workers, Sidney Altman has found some evidence for a precursor to tRNA. It is thought that this may be a punctuation or other instruction for formation of the RNA chain.

The third presentation of the morning was made by Dr. I.O. Walker (Department of Biochemistry, Oxford). He spoke on the "Structure of the Ribosome." The ribosome is a sub-cellular particle containing the enzymes which carry out cellular functions. This is true in bacteria as well as in plant and animal cells. The bacterial ribosome is a particle with a 70S molecular size. It can be reversibly dissociated into two sub-units of unequal size, one 30S and the other 50S. The 30S particle contains one 16S RNA particle and 20 or 21 protein molecules. In addition, it has about 400 magnesium ions attached to it. Its size is 110 Å by 210 Å. The 50S particle contains one 23S RNA particle and about 40 proteins. It contains about 1700 magnesium ions and has a size measuring 160 Å by 230 Å. The first problem Walker was interested in was what role

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the magnesium ion plays in stability of the ribosome particle. He found that the magnesium ions could be replaced by manganese ions in the 50S particle, without changing its structure and that the role of the magnesium ions was one of neutralizing the remaining charge on the phosphate part of nucleic acid. Walker felt that the ribosome was a spongy particle which absorbed water readily and contained magnesium ions to cover the excess phosphate reactive sites. When the 50S particle was suspended in 4 mM EDTA, it was found that it unfolded to form a 13S particle but there was no change in the RNA or protein structure, or in the combination of these structures. When the 50S particle was suspended in urea, it was changed to a 35S particle. This particle occupied three times the volume of the 50S particle. While the urea did not affect the attachment of the magnesium ions to the particle, it did alter the protein structure, bringing about dissociation of the protein from the RNA. It was concluded that the 50S particle contained an internal structure of double-stranded RNA with protein holding it in a very compact configuration. This treatment is irreversible and differs from the action of EDTA. With the latter the unfolding of the 50S particle by removal of magnesium ions is reversible. After removal of the EDTA and adding magnesium ions, the phosphate groups are neutralized and the protein is able to exert its attractive force to reform the 50S particle.

When the 50S particle was treated with endonuclease, specific for single-stranded nucleic acid, a nucleotide fraction was obtained which represented 3% of the weight of the particle, without altering appreciably the size of the 50S particle. This indicated that the particle has single-stranded RNA on its surface in addition to the internal structure of protein and double-stranded nucleic acid, with the magnesium ions distributed throughout.

When the 50S particle was further treated, the following sequence of reactions took place:



on a G-100 column the 2S fraction could be separated into:

- I. Soluble protein plus some nucleotide fraction.
- II. tRNA (double stranded) with about 60% of the nucleotide of the original particle. Average molecular weight 15,7000.
- III. Nucleotide fraction with about 40% of nucleotide of the original particle and almost all of the ATP, average molecular weight about 2000.

The 30S particle is similarly constructed, and it is thought that the two particles, the 30S and the 50S, are joined by lysine amino groups with the single-stranded nucleic acid of the 50S particle on the surface. Walker concluded that there are two moles of magnesium for each mole of 70S particle.

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In the fourth presentation Dr. G. Turnock (Department of Biochemistry, University of Leicester) spoke on "The Synthesis of Ribosomes in Bacteria." In the synthesis of 30S ribosomal subunits of *E. coli* it is known that the first detectable precursor is the 16S RNA. From this the 26S and then the 30S subunits are synthesized. This structure is heterogenic and contains 20 or 21 different proteins. The 50S ribosomal subunit is made in four stages: 23S \rightarrow 32S \rightarrow 43S \rightarrow 50S. It contains 40 or more proteins. The hold-up points in the assembly process of both these subunits may be due to the lack of a particular protein or a requirement for a steric change in one of its precursors before the next reaction will proceed.

When *E. coli* are grown in low concentrations of chloramphenicol, there is an accumulation of particles believed to be identical with the 32S and 43S precursors of the 50S subunit. Both particles contain 23S RNA. The 32S particle contains three and the 43S particle contains nine of the 19 proteins found in the 50S ribosomal subunit.

The major steps in the stage 43S \rightarrow 50S occur by the addition of the extra ten proteins.

It has been possible to prepare functional 30S subunits by combination of components in fairly high ionic strength buffer and a temperature of 40° C. It follows first-order kinetics. This has been done from 16S RNA and purified proteins (Traub and Nomura, J. Mol. Biol. 1969, 40, 391).

The fifth discussion of the Symposium was by Dr. Mary Lunt (Microbiology Unit, Department of Biochemistry, Oxford University), "The Fate of Nucleic Acids during Mixed Infections with Bacteriophages." It has been known for some time that bacteria infected with T-even phage strains may sometimes be superinfected with a second T-even phage. This phenomenon is termed temporal exclusion. When the second phage is labeled with P³², its DNA is largely converted to acid-soluble fragments by the infected cell. This super-infection DNA breakdown has occurred only when the host bacteria contained endonuclease I. Secondary infection of host bacteria deficient in endonuclease I has not resulted in solubilization of the superinfecting phage DNA. It has been suggested that temporal exclusion is caused by virus-induced changes at the cell surface. These changes prevent complete injection of the superinfecting phage DNA, the breakdown of which is a secondary process caused by the host endonuclease I.

In studying temporal exclusion further, Lunt and co-workers have reported that bacteria deficient in endonuclease I have exhibited extensive breakdown of superinfecting T2 or T4 phage DNA when infected with T5 phage. It appears that the breakdown was initiated by a T5-induced DNase. The latter enzyme is similar to that which is detectable early in T5 infection when host DNA breakdown occurs.

The addition of chloramphenicol to the culture fluids at the time the bacteria were originally infected prevented the breakdown of the super-infecting phage DNA because the production of deoxyribonuclease was inhibited.

The simultaneous infection of *E. coli* with T2 and T4 phages showed that some T2 markers were excluded from the progeny. Further experiments showed that the T4 phage induced the formation of nucleases which attacked selected regions of the T2 genome and reduced their effect for genetic transcription and for transmission to progeny phage. This work has led to the speculation that the procedures by which a specific phage genome dominates another genome may depend partly on specific recognition of the DNA involved. When this occurs, nucleases are formed which attack parts of the second genome with the result that temporal exclusion occurs.

M.A. Billeter, J.E. Dahlberg, H.M. Goodman, J. Hindley, and C. Weissmann (from Universities of Zürich, Geneva, and Bristol) authored the 6th discussion, "Studies on the Structure of the Genome of Phage Q ϕ ." Dr. J. Hindley made the presentation. The structure of the genome of phage Q ϕ is being determined by arranging conditions so that the synthesis proceeds slowly at 25° C. By stopping the process, segments of viral RNA of almost any desired length are obtained. Radioactivity may be introduced at any point. Sequence analysis is performed with the methodology of Sanger and his colleagues. The genome of phage Q ϕ is a single RNA strand containing about 3500 nucleotides. The replication of the plus strand proceeds from the 5' terminal region. The RNA strands grow in 0.06 mM phosphate buffer at the rate of 35 residues per sec at 37° C. By using 0.01 mM buffer at 25° C, the rate is reduced to 5-6 residues per sec. The ribosomes are preincubated with GTP and ATP. Then to start the synthesis, UTP and GTP are added and the reaction is allowed to proceed for 30 seconds at 25° C. In this time a segment of 160 nucleotides is obtained.

The nucleotide sequence of the first 175 nucleotides has been established; and a further segment of 150 nucleotides is now being studied. In comparing the sequences from the positive and negative sides of the 5' terminal region it was found that they matched.

The conclusions of the work to date:

- (1) No initiation triplet occurs before position 62.
- (2) At least 62 nucleotides at the 5' end and 25 nucleotides at the 3' terminus are not used to code for protein.
- (3) The sequence (PuUGGCN) required for start of synthesis of coat protein is not found within the first 300 nucleotides from the 5' end. It is suggested that the coat protein cistron is located in the middle third of the genome. This work is being continued. Hindley mentioned that he is interested in the instruction signals of the RNA.

The seventh presentation was by Prof. H.J. Subak-Sharpe (Institute of Virology, University of Glasgow), "Nucleic Acids of Animal Viruses." This was a lucid, inspiring lecture reviewing work done by the Glasgow group on developing an additional classification system for animal viruses based on doublet analysis of bases of the virus nucleic acid. This analysis gives not only the G+C content but also the frequency of

the 16 possible two-base sequences (doublets) in the nucleic acid. The method of classification provides a more detailed fingerprint of the virus than that obtained by the determination of double-strandedness or single-strandedness. By providing information on sequential arrangement of the different bases in the nucleic acid, it also overcomes the limitation of the G+C determinations which have been widely used to show species relatedness among unclassified microorganisms.

While the analytic procedure is quite involved, in general, the Kornberg enzyme (DNA polymerase) is extracted from infected cells and supplied from one labeled nucleoside triphosphate and three unlabeled triphosphates. The DNA so formed is then purified and degraded with DNase and studied for frequency distribution of the 16 possible two-base sequences. From this analysis a pattern of deviations from random (62.5 per 1000) expectation is drawn. Then the general designs of the nucleic acids from different viruses can be compared with each other and with nucleic acids from the host cells.

Doublet analysis has been carried out on about 20 animal viruses. The results show that the general designs of these viruses fall into three groups: first, all small viruses studied showed considerable resemblance to the highly characteristic general design of vertebrate host cell DNA, whether their genetic information was stored in double-stranded DNA, single-stranded DNA or single-stranded RNA. Second, all large double-stranded DNA viruses examined showed general designs which had no resemblance to host cell DNA, and which had little similarity to one another. Third, a group of intermediate-sized adenoviruses showed some resemblance to the general design of host DNA, with marked similarities to one another in doublet pattern.

This method of virus characterization presents some interesting information on possible origin of viruses and their parasitism and specificity of pathogenicity for cells of certain species.

The eighth presentation was by Prof. H. Harris (Sir William Dunn School of Pathology, Oxford), "The Expression of Genetic Information by Somatic Cell Nuclei." This was a well-done exposition of work carried out by fusion of bird red-blood cell nuclei into human or mouse nucleated cells. The synthesis of RNA and proteins by the cell which received the new nucleus was studied.

It has been known that the nucleus of a fowl red-blood cell is not active in its normal cell habitat. However, when it was placed inside a mammalian cell, it became activated and started functioning. Autoradiographs showed that when radioactive precursors were added, RNA was synthesized by the fowl nucleus. However, the nucleus did not contain a nucleolus and synthesis of chicken protein could not be detected. When mitosis occurred, the chicken nucleus was fragmented and lost.

By blocking mitosis with X-rays, the chicken nucleus continued to grow. It now developed a nucleolus and chicken specific protein could

be detected. When the mouse A9 cell was studied, it was found that normally it could not incorporate tritiated hypoxanthine. When the fowl nucleus was inserted, hypoxanthine was not incorporated until the nucleoli were formed. Then six days after fusion, hypoxanthine was incorporated and evidence of inosinic acid phosphorylase was definitely obtained. It was also found that the RNA in the cell was polydisperse until the nucleolus was formed, then it had the normal ribosome nucleic acid size. Apparently the fowl nucleus started functioning after the normal cell nucleus had been killed by the X-ray. The fowl nucleus survived because it was resistant to X-ray treatment before activation. These experiments have indicated that the RNA, which carries the information for synthesis of proteins and structural RNA components in the ribosomes, passes from the nucleus to the cytoplasm either as one structure or as related structures.

ORIGINAL PAPERS

The program of original papers which were presented on the second day was as follows:

Session A

1. A. Zaritsky (Department of Genetics, University of Leicester) "Rate of DNA replication in thymine-requiring strains of Escherichia coli."
2. I.R. Beacham (Department of Genetics, University of Leicester) "The thymidine triphosphate pool in wild-type and thymine-requiring strains of Escherichia coli K12 and 15."
3. D.H. Williamson, N.G. Maroudas, and D. Wilkie (National Institute for Medical Research, Mill Hill and Department of Botany, University College, London) "Induction of the cytoplasmic petite mutation in Saccharomyces cerevisiae."
4. H.G. Nandadase (Department of Genetics, University of Leicester) "Properties of male derivatives of Escherichia coli mutants defective in initiation of chromosome replication."
5. J. Collins (Department of Genetics, University of Leicester) "The Timing of episomal F replication in Escherichia coli at different growth rates."
6. M.R. Blundell and D.G. Wild (Microbiology Unit, Oxford University) "The accumulation of ribosome precursors during inhibition by cobalt chloride."
7. M.R. Blundell and D.G. Wild (Microbiology Unit, Oxford University) "Altered ribosomes after inhibition of Escherichia coli by rifampicin."
8. Q. Mehdi and D.G. Wild (Microbiology Unit, Oxford University) "Synthesis of ribosomes in a mutant of Escherichia coli."

9. G.K. Darby (Department of Virology, University of Birmingham), L.B. Dumas (Department of Biological Sciences, Northwestern University, Illinois), and R.L. Sinsheimer (Division of Biology, California Institute of Technology) "Pyrimidine sequences in the complementary strand of the replicative form of bacteriophage ϕ X 174 DNA."
10. J.P. Gratia (Laboratory of Microbiology and Hygiene, University of Liège, Belgium) "Abnormal prophage excision in terminal deletion lysogens of phage ϕ 80. Evidence for a chromosomal locus of sensitivity to the K-restriction near the prophage attachment site."
11. R.N. Strange and H. Smith (Department of Microbiology, University of Birmingham) "Partial purification and properties of a fungal growth stimulant in anthers which predisposes wheat to attack by Fusarium graminearum."
12. P.N. Hobson and C.S. Stewart (Rowett Research Institute, Bucksburn) "Growth of two rumen bacteria in mixed culture."
13. M.P. Stratford and A.J. Griffiths (Department of Microbiology, University College, Cardiff) "Excystment of Hartmannella castellani."
14. J.G. Anderson and J.E. Smith (Department of Applied Microbiology, University of Strathclyde) "A fermentor culture system for sporulation studies on Aspergillus niger."
15. C.G. Orpin and G.S. Coleman (Department of Biochemistry, A.R.C. Institute of Animal Physiology, Babraham, Cambridge) "The cultivation of 'ovals' from the ovine rumen."
16. B.M. Mackey and J.G. Morris (Department of Biochemistry, School of Biological Sciences, University of Leicester) "Ultrastructural changes during sporulation in Clostridium pasteurianum."
17. D. Gaudie and H. Tristram (Department of Botany and Microbiology, University College, London) "Proline biosynthesis: regulation by a positive control mechanism?"
18. D.A. Stafford and A.G. Callely (Department of Microbiology, University College, Cardiff) "Properties of a pyridine degrading organism"
19. Lynda M. Sapshead and J.W.T. Wimpenny (Department of Microbiology, University College, Cardiff) "The effect of oxygen and nitrate on respiratory systems in Micrococcus denitrificans."
20. J.W.T. Wimpenny (Department of Microbiology, University College, Cardiff) "Oxygen-induced proton pulses in various species of bacteria"

Session C

21. Janet E. Hutchinson, B.W.J. Mahy and R.D. Barry (Department of Pathology, University of Cambridge) "The nature of the ribonucleic acid synthesized in vitro by Sendai virus-induced ribonucleic acid-dependent ribonucleic acid polymerase."

22. P.A. Bromley and R.D. Barry (Department of Pathology, University of Cambridge) "Studies on the nature of influenza virus ribonucleic acid."
23. Q.R. Miranda and T.S.L. Beswick (Department of Bacteriology and Virology, University of Manchester) "Anti-interferon factors in the digestive tract."
24. B.G. Spratt and R.J. Rowbury (Department of Botany and Microbiology, University College, London) "A mutant in the initiation of DNA synthesis in Salmonella typhimurium."
25. B.G. Spratt and R.J. Rowbury (Department of Botany and Microbiology, University College, London) "Production of DNA-less cells in a mutant of Salmonella typhimurium."
26. J. Semal and J. Kummert (Faculté des Sciences agronomiques, Gembloux, Belgium) "Synthesis of double-stranded and single-stranded RNA by cell-free extracts of barley leaves infected with bromegrass mosaic virus."
27. G.W. Notani (Institute of Molecular Biology, Geneva, Switzerland) "RNA synthesis during growth of phage T4."

As noted above, Session C was somewhat shorter than Sessions A or B. This was due to time out for a short business meeting of the Society.

There were a number of interesting papers among those listed above. Several are reviewed here:

In describing the production of DNA-less cells in a mutant of Salmonella typhimurium (Paper No. 25), Spratt and Rowbury referred to work of Hirota, et al. (J. Mol. Biol. 1968, 35, 175) and Inouye (J. Bact. 1969, 99, 842) who reported isolation of certain E. coli mutants that continue cell division in the absence of DNA synthesis. Spratt found a temperature-sensitive mutant of S. typhimurium which was unable to initiate new rounds of DNA replication when shifted from growth at 25° C to 35° C. After the shift, the cells divided once or twice depending upon the growth medium. The divisions resulted in distribution of the chromosomes in reduced amount in each daughter cell. On continued incubation, the cells elongated to form filaments. After about an hour, cell division commenced at the ends of the filaments and small cells were formed. Each end of the filament produced more than one small cell. These cells were isolated on a sucrose gradient. They contained less than 10% of the DNA of normal cells and synthesized no protein, RNA or DNA. Phage P22 was unable to develop in these cells. The addition of nalidixic acid at the time of the shift from 25° C to 35° C resulted in abrupt cessation of cell division and no small cells were formed.

"Partial Purification and Properties of a Fungal Growth Stimulant in Anthers which Predisposes wheat to Attack by Fusarium graminearum" (Paper No. 11). In this study Strange and Smith found a growth stimulant

in the anthers of wheat which appeared to play a decisive role in the establishment of infection of the wheat head by Fusarium graminearum. The presence of the growth stimulant was demonstrated by measuring the radius of growth of the fungus on agar plates containing extracts of the plant in wells in the agar. The maximum stimulation produced growth 70% greater than that in the control medium. Extracts of the anthers were more active than extracts from other parts of the plant. An active extract was prepared by chromatography on carboxymethylcellulose and by fractionation with alcohol. The active material was soluble in alcohol, was a basic substance which did not show specific absorption of uv light and had a low molecular weight (i.e., it dialyzed through cellophane (Visking) tubing).

"Excystment of Hartmannella castellanii," (Paper No. 13) by Stratford and Griffiths. The process of excystment of the soil amoeba Hartmannella castellanii was studied. The cysts used in this study maintained their viability after exposure to low temperature and extremely dry conditions (over calcium chloride). Temperatures in excess of 60° C have rendered the cysts non-viable. Excystment under axenic conditions was induced by suspending the cysts in 4% to 9% peptone solution. Although simple media and bacterial extracts are less effective, in general, cysts exhibit the same nutrient requirements and temperature and pH optima for excystment as for growth. The time required for excystment was somewhat variable, but the process usually took three to five days and was relatively synchronous within each culture.

With H. castellanii the process of excystment consisted of at least three stages: (a) initiation phase, not much was seen during this phase, although cysts suspended in 0.15 M $MgCl_2$ showed vacuolation of the cytoplasm. This was the first sign of growth; (b) pre-emergence phase -- during this time the cysts showed some increase in dry weight, there was an increase in total acid phosphatase activity and there was a slight increase in oxygen consumption; (c) the outgrowth phase -- during this phase amoebae emerged and appeared in the culture. After outgrowth, structurally perfect but empty cysts remained, so that apparently the emergence was through a pore in the cyst wall.

CONCLUSION

Each meeting of the Society is held at a different place. This provides opportunities for members to become acquainted with workers at the various laboratories throughout Britain. Each meeting includes a one- or two-day symposium, as well as sessions for the reading of original papers. The subject of the Symposium is usually related to the work being conducted in the institution where the meeting is held. Abstracts of each original paper and of the two one-day symposia are published in the Journal of General Microbiology. Complete texts of the presentations made at the two-day symposium in April are published each year by the Society as a Symposium Volume. To date 20 volumes of symposia have been published.

The Society also takes an active interest in international aspects of microbiology. Recently a North West European Group of Microbiological Societies was formed, including the Societies of Denmark, Finland, Great Britain, Norway, Sweden and The Netherlands. Meetings are held annually in one of the member countries, except during the year when an International Microbiological Congress meets.

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13. ABSTRACT The regular fall meeting of the Society brought together scientists, teachers and students from British universities. The feature of the meeting was a Symposium on Function and Metabolism of Nucleic Acids which presented various aspects of synthesis and structure of ribose nucleic acids and their configuration in bacterial ribosomes. The fate of deoxyribonucleic acids during mixed infections with bacteriophages was reviewed. Also included were a fascinating description of the expression of genetic information by avian red blood cell nuclei when introduced into nucleated mouse cells, and the use of nearest neighbor base analyses for characterizing nucleic acids of viruses. In addition to the Symposium, 27 original papers were read in double sessions occupying one day. The papers covered work with viruses, bacteria, fungi and protozoa.		

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There is no limitation on the length of the abstract. However, the suggested length is from 150 to 225 words.

14. **KEY WORDS:** Key words are technically meaningful terms or short phrases that characterize a report and may be used as index entries for cataloging the report. Key words must be selected so that no security classification is required. Identifiers, such as equipment model designation, trade name, military project code name, geographic location, may be used as key words but will be followed by an indication of technical content. The assignment of links, roles, and weights is optional.

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